9. EXPERIMENTAL STUDIES ON THE MONOCOTYLEDONOUS MONOSULCATE POLLEN GRAINS

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Abstract

Pollen grains of *Lilium candidum* L. were the subject of our investigations. Fresh and heated pollen grains at 200 °C during 10 min., 1 hour, 5, 10, 25, 50 and 100 hours were investigated with the light microscope. Alterations in the polar axis and the P/E ratio were investigated. In general the pollen grains of this species are resistant, no taxonomically important alterations were observed as a consequence of the high temperature effect.

Key words: Palynology, recent, *Lilium candidum*, high temperature effect.

Introduction

The monosulcate form of the pollen grains is very important from the point of view of the angiosperm evolution. In general it may be taken as an early form. The peculiar reticulate type of the *Lilium* pollen grain is also a primitive caracteristic feature. Following BRENNER (1963) the fossil form-genus *Liliacidites* is represented in the Barremian-Aptian with two species (*peroreticulatus* and *reticulatus*). LAING (1976) established, that the monosulcate forms with clavate and/or reticulate sexines may be the first definitive angiosperm pollen. The pollen grains of the recent taxa of the Liliaceae were the subject of several different kinds of investigations. The basic LM morphological papers, among others, supported the role of the pollen morphology in the solution of taxonomic problems. For example: SHARMA (1967/68), SCHULZE (1975a,b, 1978a,b, 1980a,b), CHANDA and GHOSH (1976), TAKAHASHI (1987a,b), TAKAHASHI and SOHMA (1982, 1983), DIAZ LIFANTE, DIEZ and FERNÁNDEZ (1990) and KOSENKO (1999). Using the TEM method MEYER and YAROSHEVSKAYA (1976) established that the lamellar nexine in Liliaceae and Cyperaceae suggest a common ancestry. Following DICKINSON (1970), in *Lilium* the primexine is radially lamellate in probacules and vesiculate in the development of the muri. Further data concerning the development of the Liliaceae pollen grains was given by TAKAHASHI (1987), and SOHMA and TAKAHASHI (1982). HESLOP-HARRISON (1973) established radical chages in *Lilium* cytoplasmatic membranes of the meiocyte with a prophase decline in ribosome number. Following KNOX (1973), the thin intines of Liliaceae and Amaryllidaceae show less enzymatic activity, although in the large grains of *Lilium* spp. the hydrobases are readily detectable in the colpal zone. The protoplast of *Lilium longiflorum* THUNB. and in vitro regenerations of the cellulozic walls were investigated by MIKI-HIROSIGE, NAKAMURA and TANAKA

(1988). There are several papers on the orbicules of the pollen grains of *Lilium* (CLÉMENT and AUDRAN, 1992, 1993a,b,c).

The secretion from the pistil of *Lilium longiflorum* was published by MIKI-HIROSIGE, HOEK and NAKAMURA (1987). MIKI-HIROSIGE (1961) investigated the pollen germination and pollen tube growth in pistil, stigma, style and ovary slices. ROSEN (1973a), p. 177, established the following: "*Lilium longiflorum* pollen cytoplasm possesses numerous lipid droplets but lack starch". The metabolism of germinating *Lilium* pollen was described by DAVID (1973). DASHEK, HARWOOD, and ROSEN (1973) pointed out the significance of the wall-bound hydroxyproline-containing glycoproteide in *Lilium* pollen tube elongation.

The first data on the chemistry of the pollen wall was published by JOHN (1814) from a Liliaceae pollen grain (*Tulipa*). *Lilium henryi* THUNB. was the subject of the study of BROOKS and SHAW (1968, 1973, 1978). It was established, that the precursors of the sporopollenin are β-caroten and its esters. The precursor importance of the phenylalanine in the pollen grains of the genus *Tulipa* was demonstrated by RITTSCHER, GUBATZ and WIERMANN (1987). SOUTHWORTH (1985, 1986) acetolyzed exines of *Lilium longiflorum* THUNB. partially extracted with hot 2-aminoethanol. The residual material was investigated with the TEM method.The lattice-like substructure of interconnected granules was composed of different kinds of polygons. Previously we investigated the high temperature effect on monosulcate, tricolpate and tricolporate pollen grains (KEDVES et al., 1993) and it was established, that after heating the monosulcate angiosperm pollen grains, such as *Magnolia, Allium* and *Chamaedorea*, are similar to early Mesozoic gymnosperm pollen grains.

The aim of this paper is to show new results on the secondary alterations of the monosulcate monocotyledonous pollen grains.

Materials and Methods

The material investigated was collected by Miss. A. HEGEDÜS, and I. OLÁH in the Botanical Garden of the University of Szeged, on the 9 June 1990. The pollen material was frozen at -20 °C. The experiments were started on 21 January 1992. Fresh (1285) and heated pollen grains were the subject of our investigations. Temperature: 200 °C, Length of time: 10 minutes (1286), 1 hour (1287), 5 hours (1288), 10 hours (1289), 25 hours (1290) and 50 hours (1291), 100 hours (1292). The distribution in percentages of the P/E ratio and the variation of the polar axis were measured.

Results

Pollen grains, as well established during previous investigations, are monosulcate and in surface sculpture, characteristically reticulate (Plate 9.1., figs. 1-3). The mesh of the reticula in the inter-apertural area (proximal pole) is about 3.6-8.4 μm (Plate 9.1., fig. 3), in the apertural area it is smaller 1.6-2.8 μm (Plate 9.1., figs. 1,2). The amb of the fresh pollen grains is spindle shaped (Plate 9.2., fig. 1). The polar axis varies from 62.5 μm to 100.0 μm (Table 9.1.), the P/E ratio is 1.1-2.0, maximum, 28.0% at 1.3. (Table 9.2).

Pollen grains heated for 10 minutes (Plate 9.2., fig. 3, table 9.1., 9.2)

The length of the polar axis increased from 70.0 μm to 122.5 μm, 11.5% at 95.0.μm, 10.5% at 105.0 μm. Important changes happened to the P/E ratio, this value is 2.0, in the greatest part of the pollen grains (15.5%).

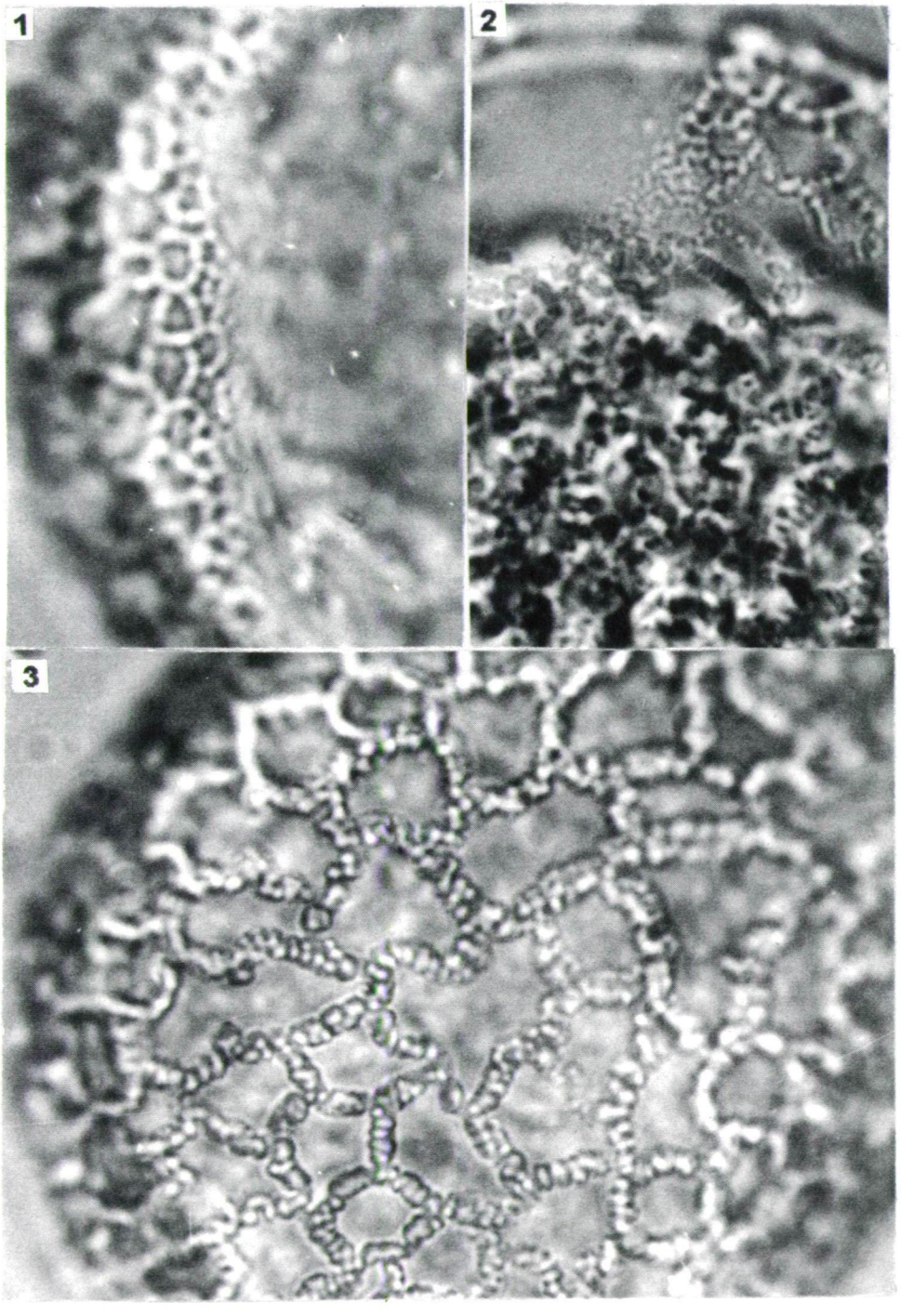

Plate 9.1.

Plate 9.2.

Plate 9.1.

1-3. *Lilium candidum* L., fresh pollen grains, 2500x.
1,2. Detail from the apertural area. The reduced diameter of the mesh of the reticula is well illustrated.
3. Surface ornamentation of the pollen grain on the proximal surface.

Plate 9.2.

1-12. *Lilium candidum* L. 750x.
1,2. Fresh pollen grains.
3. Pollen grain heated for 10 minutes.
4. Pollen grain heated for 1 hour.
5. Pollen grain heated for 5 hours.
6. Pollen grain heated for 10 hours.
7,8. Pollen grains heated for 25 hours.
9,10. Pollen grains heated for 50 hours.
11,12. Pollen grains heated for 100 hours.

Pollen grains heated for 1 hour (Plate 9.2., fig. 4, table 9.1., 9.2)

The length of the polar axis is nearly the same as in the previous experiment, from 67.5 µm to 120.0 µm, maximum (11.5%) is at 102.5 µm. The P/E ratio varies from 1.2 to 2.6, maximum (13.5%) at 2.2.

Pollen grains heated for 5 hours (Plate 9.2., fig. 5, table 9.1., 9.2)

The length of the polar axis varies from 70.0 µm, to 115.0 µm, the maximum is a little decreased, 15.0% at 100.0 µm. The P/E ratio is also reduced in contrast to the previous experiment, 18.5% is at 1.9.

Pollen grains heated for 10 hours (Plate 9.2, fig. 6, table 9.1, 9.2)

The length of the polar axis is 70.0 µm to 120.0 µm,the maximum is 87.5 µm (12.0%) therefore the previously mentioned trend continuted. The maximal value of the P/E ratio (1.8) is 14.5%.

Pollen grains heated for 25 hours (Plate 9.2., figs. 7,8, table 9.1, 9.2)

The length of the polar axis is 70.0 µm to 117.5 µm, the maximum (12.0%) is at 100 µm. This is a quite similar to the previous experiment. P/E ratio from 1.1 - 2.2, maximum (14.5%) at 1.9.

Pollen grains heated for 50 hours (Plate 9.2., figs. 9,10, table 9.1., 9.2)

Polar axis from 60.0 µm to 112.5 µm, maximum (11.0%) at 95.0 µm. High percentage (105%) was measured at 82.5 µm, a trend of reduction in consequence of the heating may be established.The P/E ratio maximum is identical with the previous experiment, 17.5% at 1.9.

Pollen grains heated for 100 hours (Plate 9.2., figs. 11,12, table 9.1., 9.2)

A remarkable diminution was observed in the length of the polar axis. Percentages over 10% were observed at 80.0 - 87.5 µm, altogether 47.5%.The P/E ratio is nearly the same as previously. This character has not changed significantly from the heating for 5 hours.

	60.0	62.5	65.0	67.5	70.0	72.5	75.0	77.5	80.0	82.5	85.0	87.5	90.0	92.5	95.0	97.5	100.0	102.5	105.0	107.5	110.0	112.5	115.0	117.5	120.0	122.5
1285		0.5	0.5	1.0	5.0	5.5	12.0	17.0	18.5	13.0	12.0	9.5	2.5	1.5	0.5		1.0									
1286					0.5	1.5	1.5	2.0	1.5	3.0	5.0	6.5	7.5	7.5	11.5	6.5	8.0	9.0	10.5	6.0	5.0	3.0	2.5		0.5	1.0
1287				0.5	0.5	0.5	0.5	2.0	2.5	4.0	3.0	8.0	6.0	3.0	9.0	8.0	8.5	11.5	8.0	6.5	5.0	10.0		2.0	1.0	
1288					0.5	1.5	1.5	3.5	3.5	3.0	4.0	7.5	9.0	9.0	10.0	6.5	15.0	4.0	6.5	7.0	5.0	2.0	1.0			
1289					0.5	0.5	4.5	3.5	3.0	6.0	4.0	12.0	5.5	7.0	8.0	7.5	11.5	6.5	7.5	5.5	1.0	1.0	4.0	0.5	0.5	
1290					1.0	1.5	4.5	5.0	2.0	4.0	6.5	9.5	5.5	7.5	11.5	7.0	12.0	3.5	6.5	6.0	2.0	3.0	1.0	0.5		
1291	0.5	0.5	1.0	2.5	4.0	2.5	6.5	3.0	7.0	10.5	12.5	13.5	6.0	6.5	11.0	2.0	2.5	3.5	1.0	2.5		1.0				
1292	0.5	0.5	1.0	2.5	7.0	4.0	9.0	5.0	11.5	11.5	14.0	11.5	8.0	5.0	3.0	4.5	1.0			0.5						

Table 9.1.

The percentages of the length of the polar axis of the pollen grains. Explanation: 1285, fresh pollen grain, 1286, pollen grains heated for 10 minutes, 1287, pollen grains heated for 1 hour, 1288, pollen grains heated for 5 hours, 1289, pollen grains heated for 10 hours, 1290, pollen grains heated for 25 hours, 1291, pollen grains heated for 50 hours, 1292, pollen grains heated for 100 hours.

P/E ratio	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9
Experiment No																			
1285	12.0	23.5	28.0	19.0	9.0	5.0	1.5	1.0	0.5	0.5									
1286	1.0	2.0	1.5	2.5	7.0	6.5	11.5	13.0	12.5	15.5	11.5	6.5	4.0	2.5	2.0		0.5		
1287		2.5	1.0	1.5	3.0	3.0	11.5	13.0	7.5	21.0	10.5	13.5	4.5	4.0	2.0	1.5			
1288		0.5	4.0	4.0	1.5	7.0	7.0	9.0	18.5	15.0	12.5	13.5	4.0	1.5	1.5	0.5			
1289	0.5	1.5	2.5	5.0	8.0	3.5	9.0	14.5	13.5	10.0	12.5	10.0	6.0	2.0	1.0				0.5
1290	0.5	6.0	6.5	4.0	7.0	6.5	11.5	11.5	14.5	11.0	10.0	7.0	4.0						
1291		3.0	1.5	4.0	5.5	9.0	7.5	9.5	17.5	15.0	11.5	8.0	5.0	2.5	0.5				
1292	0.5	1.0	3.0	4.5	2.5	9.5	11.0	12.5	13.0	11.0	12.0	6.0	8.5	2.5	2.0	0.5			

Table 9.2.

The percentages of the P/E ratio of the pollen grains. Explanation 1285, fresh pollen grains. 1286, pollen grains heated for 10 minutes, 1287, pollen grains heated for 1 hour, 1288, pollen grains heated for 5 hours, 1289, pollen grains heated for 10 hours, 1290, pollen grains heated for 25 hours, 1291, pollen grains heated for 50 hours, 1292, pollen grains heated for 100 hours.

Discussion and Conclusions

1. In the introduction, an attempt was made to review the most important fields of investigation concerning monosulcate pollen grains, in particular the Liliaceae. The phylogenetic importance of this kind of pollen grains is remarkable, cf. M. VAN CAMPO (1967, 1976).

2. A long time ago KIRCHHEIMER, 1933 that during the fossilization process the high temperature may provoke secondary alterations. In order to interpret the palynological data from metamorphic rocks so called model experiments are necessary.

3. The pollen grains of *Lilium* seem to be morphologically resistant. Important changes in the quantitative morphological characteristic features happen after 10 minutes of heating. The polar axis and the P/E ratio of fresh and the briefly heated pollen grains are extremely different. The averages of the polar axis and the P/E ratio are summarized as follows.

Duration of heating	Polar axis	P/E ratio
0	80.125	1.3225
10minutes	96.95	1.875
1hour	98.325	1.955
5hours	95.125	1.92
10hours	94.35	1.88
25hours	93.275	1.78
50hours	86.225	1.873
100hours	82.3	1.89

Based on these data no important changes may be observed in the quantitative data of the heated pollen grains, in contrast to the evaluation of the detailed data, see Table 9.1., and 9.2.

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